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REVIEW

Aquatic acidification: a mechanism underpinning maintained oxygen transport and performance in fish experiencing elevated carbon dioxide conditions

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ABSTRACT

Aquatic acidification, caused by elevating levels of atmospheric carbon dioxide (CO_2), is increasing in both freshwater and marine ecosystems worldwide. However, few studies have examined how acidification will affect oxygen (O_2) transport and, therefore, performance in fishes. Although data are generally lacking, the majority of fishes investigated in this meta-analysis exhibited no effect of elevated CO_2 at the level of O_2 uptake, suggesting that they are able to maintain metabolic performance during a period of acidosis. Notably, the mechanisms that fish employ to maintain performance and O_2 uptake have yet to be verified. Here, we summarize current data related to one recently proposed mechanism underpinning the maintenance of O_2 uptake during exposure to aquatic acidification, and reveal knowledge gaps that could be targeted for future research. Most studies have examined O_2 uptake rates while fishes were resting and did not calculate aerobic scope, even though aerobic scope can aid in predicting changes to whole-animal metabolic performance. Furthermore, research is lacking on different age classes, freshwater species and elasmobranchs, all of which might be impacted by future acidification conditions. Finally, this Review further seeks to emphasize the importance of developing collaborative efforts between molecular, physiological and ecological approaches in order to provide more comprehensive predictions as to how future fish populations will be affected by climate change.

KEY WORDS: pH, Teleost, Metabolic rate, Oxygen uptake, Root effect, Climate change, Acidification, Freshwater, Ocean

Introduction

The concentration of atmospheric carbon dioxide (CO_2) has been increasing at unprecedented rates since pre-industrial times to approximately ∼400 ppm in 2015 (Dlugokencky and Tans, 2015), the highest level in the atmosphere in 800,000 years (Lüthi et al., 2008). Since the Industrial Revolution, approximately 30% of the excess CO_2 in the atmosphere has been absorbed by the oceans (Sabine et al., 2004), which, owing to their expansive surface area, experience a parallel increase in the partial pressure of CO_2 (P_{CO_2}) with the atmosphere (Doney, 2010). If the current trajectory is maintained, increases in atmospheric CO_2 levels are predicted to exceed 900 ppm by 2100 (Meinshausen et al., 2011). Increasing P_{CO_2} of marine systems is often accompanied by a decrease in pH of the water, termed ocean acidification (OA) (Doney, 2010). Although it is known that ocean P_{CO_2} is increasing and pH is

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decreasing, reliable *in situ* records of these parameters are lacking (Degrandpre et al., 1995; Hofmann and Todgham, 2010). Moreover, if we continue along the current trajectory predicted by the Intergovernmental Panel on Climate Change IS92a 'business as usual' emissions scenario for the year 2100, the ocean pH is predicted to decrease by 0.4 pH units by 2100 (Meinshausen et al., 2011; Pörtner et al., 2014). Predictions for future conditions in marine systems are affected by the composition of seawater, which, when compared with all other aquatic systems, is extremely constant, ranging by ±7% in salinity (Berner and Berner, 2012) and by less than 1% with respect to ion ratios (Millero et al., 2006). Unlike the many predictions and models depicting future ocean scenarios, those for freshwater systems are less clear.

Freshwater chemistry is affected by a multitude of factors, and the P_{CO_2} varies greatly within and between different systems and locations. Cole et al. (2007) found that the current P_{CO_2} range in lakes varies from 3.1-fold below to 16-fold above current atmospheric levels, with a mean of ∼1000 µatm. Larger lakes that might be deemed similar to marine systems in terms of the surface area in contact with the atmosphere tend to remain close to atmospheric levels (Cole et al., 2007). However, smaller systems (e.g. rivers and streams) can vary depending on surrounding terrestrial ecosystems, precipitation, surface area, residence time (see Glossary), etc. (Butman and Raymond, 2011; Kokic et al., 2015). Raymond et al. (2013) found that, in over 6700 stream and river systems assessed at a global scale, 95% had a median P_{CO_2} level higher than current atmospheric levels (∼400 ppm). Given that freshwater systems are so variable and affected by many different environmental and anthropogenic factors, it is difficult to predict how the rising atmospheric CO_2 will influence future freshwater systems. A case study on the Laurentian Great Lakes in North America concluded that larger systems would experience a future increase in P_{CO_2} similar to that expected for the oceans (Phillips et al., 2015). However, future trends are difficult to predict in smaller systems. What is clear, however, is that, along with increases in atmospheric CO_2, variations and/or increases in P_{CO_2} will continue to occur in all aquatic systems (Hasler et al., 2016).

Although both long-term data on P_{CO_2} and pH for marine systems and predictions for freshwater systems are lacking, elevated P_{CO_2} (hypercapnia; see Glossary) is expected to have detrimental impacts on an array of aquatic organisms, highlighting the need for more research and reliable measurements and/or predictions. Some observed detrimental effects of aquatic acidification (AA; see Glossary) upon both freshwater and marine calcifying molluscs include: reductions in calcification rates, growth, reproduction and immune function, as well as alterations in ion-exchange mechanisms and behaviour (Bibby et al., 2008; Hannan et al., 2016; Michaelidis et al., 2007; Waller et al., 2017; Watson et al., 2013). The tissues and fluids of many aquatic organisms (i.e. molluscs and

Glossary

Aerobic scope (AS)
The total aerobic energy available to an organism above basic maintenance costs for life-history processes, such as growth and reproduction.

Apex predator
A predator at the top of the food chain.

Aquatic acidification (AA)
Elevated P_{CO_2} levels above ambient concentrations but ≤2000 µatm.

Carbonic anhydrase (CA)
Enzyme that catalyses the rapid conversion of carbon dioxide and water to bicarbonate and protons (or vice versa).

Chondrichthyan
Organisms of the class that contains cartilaginous fishes (i.e. sharks, skates and rays).

Elasmobranch
Organisms of a subclass of Chondrichthyes encompassing cartilaginous fishes (i.e. sharks, skates and rays).

Haemoglobin (Hb)
Protein in the red blood cell responsible for the transportation of oxygen throughout the body of vertebrates.

Housekeeping
Maintenance of basic physiological function.

Hypercapnia
Elevated CO_2 levels.

Ionocyte
Type of cell found in fish gills that plays a role in ion exchange with the environment.

Meso-predator
A predator in the middle of the food chain, which preys on smaller animals.

Ray-finned fish
All bony fishes except coelacanth and lungfishes.

Residence time
The average amount of time that something (i.e. water) spends in a particular system.

Root effect Hbs
Unique Hb to fishes that are highly pH sensitive.

fishes) become acidified when these organisms are exposed to elevated P_{CO_2} in their water because of the rapid diffusion of uncharged CO_2 molecules across cell membranes (Ishimatsu et al., 2005). Marine molluscs represent one of the most studied taxa with respect to AA, and molluscs are assumed to be intolerant to elevated P_{CO_2} when compared with fishes. Freshwater and marine fishes can physiologically compensate for an extreme acidosis (>10,000 µatm) within hours to days (Fig. 1), which has led many to suggest that fish are resilient to elevated environmental P_{CO} (Heuer and Grosell, 2014). However, negative impacts have been consistently observed in marine fishes at the level of behaviour, even at relatively low levels of P_{CO_2} (Briffa et al., 2012; Heuer and Grosell, 2014; Munday et al., 2012). Additionally, although far less studied, there is also the potential for freshwater fishes to experience behavioural alterations due to elevated P_{CO_2} (Tix et al., 2017). These behavioural impairments demonstrate that, despite fish being efficient acid–base regulators, they may not be as tolerant to an acidosis as previously predicted. Indeed, the capacity for acid–base regulation has recently been linked to the observed behavioural impairments related to an ion imbalance at the $GABA_A$ receptor (Fig. 1; also see Heuer et al., 2016; Nilsson et al., 2012).

Behaviour is not the only potential negative impact fishes may incur as a result of acidification – an additional stress could come from the energy requirements of acid–base regulation (Fig. 1), which is known to be metabolically costly, even under steady-state

conditions (Baker and Brauner, 2012). However, even though there is a prediction for increased energy demand with acid–base regulation under acidification conditions, various fish species have exhibited an array of metabolic responses to elevated P_{CO_2}, ranging from a decrease (Munday et al., 2009) to increases (Couturier et al., 2013; Rummer et al., 2013a) in metabolic performance. Additionally, the responses at the level of aerobic scope (AS; see Glossary) – the total aerobic energy available above basic maintenance costs – to elevated P_{CO_2} have been varied as well. One explanation for this variability may arise upon closer examination of model teleost fishes.

Studies on model teleost fishes, such as the salmonids, have identified a mechanism responsible for enhanced tissue oxygen (O_2) delivery when fish are exposed to elevated P_{CO_2} (Alderman et al., 2016; Rummer and Brauner, 2011; Rummer et al., 2013b). Most teleost fishes possess extremely pH-sensitive haemoglobins (Hb; see Glossary), where reductions in pH not only reduce Hb–O_2 affinity (Bohr effect) but also greatly reduce Hb–O_2 carrying capacity (Root effect). In the presence of an acidosis, the extracellular pH (pH_e) decreases initially (Baker et al., 2009), and many fish species release catecholamines (e.g. adrenaline, noradrenaline) into the circulation to activate sodium/proton (Na^+/H^+) exchange (βNHE) on the surface of the red blood cells (RBCs) to remove H^+ and elevate intracellular pH (Rummer et al., 2013b) (Fig. 1). Elevated RBC pH increases Hb–O_2 affinity, and therefore O_2 uptake at the gill and subsequent tissue O_2 delivery is safeguarded. To enhance O_2 delivery at targeted locations (e.g. red muscle, heart, etc.), carbonic anhydrase (CA; see Glossary) that is plasma-accessible in those select locations short-circuits this protective mechanism. The result is a re-acidification of the RBC, decreased Hb–O_2 affinity, and O_2 release to the tissue (Rummer et al., 2013b) (Fig. 1). In salmonids, this mechanism might also be operating under a very mild acidosis as well, one in which catecholamines are not released, perhaps through a more general 'housekeeping' (see Glossary) NHE (Rummer et al., 2013b). Regardless of the mechanism, the link between enhanced O_2 delivery and enhanced performance has not yet been made. Additionally, it is unclear whether this mechanism for enhanced O_2 delivery via Root effect Hbs (see Glossary) and plasma-accessible CA exists in more derived teleosts (i.e. those that appeared more recently on the evolutionary time scale; e.g. coral reef fishes) and whether it can be initiated under a low-level acidosis, such as what has been used in OA studies (~850 to 1000 µatm), where maintained or even increased AS has been documented (reviewed in Heuer and Grosell, 2014). Thus, if enhancing O_2 delivery to tissues during elevated P_{CO_2} translates to maintained or increased AS across a wide range of fishes, there could be a mechanistic explanation to what has been documented in the literature, which could also aid in predicting how fishes will respond to AA into the future.

Bearing the above in mind, our aim here is to highlight potential mechanisms for changes in O_2 transport that could underpin changes in performance in fish in response to elevations in P_{CO_2}. Past reviews on O_2 transport have focused on OA without taking freshwater systems into account, and so both marine oceanic and freshwater fishes, and their respective findings to date, will be discussed here. Furthermore, although elasmobranchs (see Glossary) are not known to possess the Root effect Hbs that are unique to teleosts, some elasmobranch species have been found to be unaffected at AA-relevant P_{CO_2} levels in terms of AS and metabolic performance; therefore, potential mechanisms for maintaining O_2 transport in elasmobranchs under elevated P_{CO_2} will also be discussed. For this Review, we have focused on experiments that manipulated water P_{CO_2} and measured the metabolic responses of fishes. We have reviewed existing studies
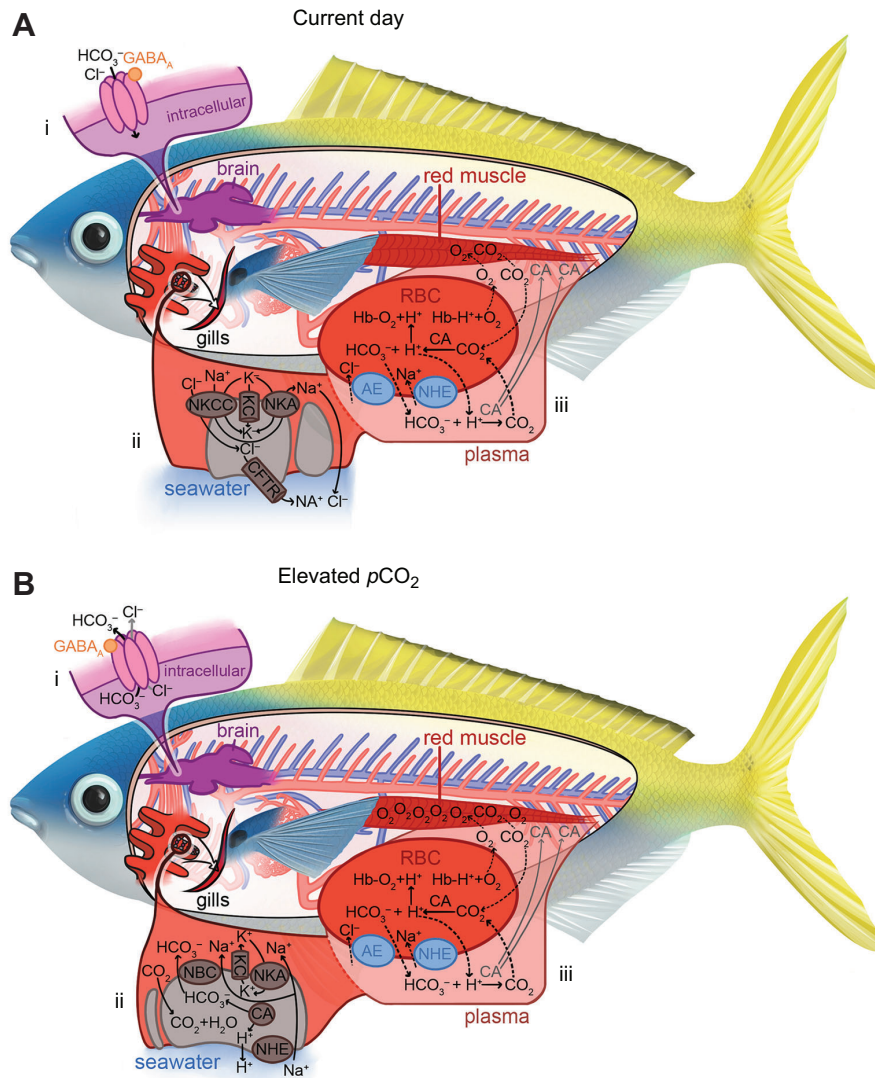
Fig. 1. Physiological mechanisms of fishes in the marine environment when exposed to current-day partial pressures of carbon dioxide (P_{CO_2}) and during times of elevated P_{CO_2} (780–2000 µatm). During exposure to current-day conditions (Ai), the concentrations of intracellular chloride (Cl^-) are less than extracellular Cl^-, and, at the brain, when Cl^- ions move into the cell via the gamma-aminobutyric acid type A ($GABA_A$) receptors, the neuron becomes hyperpolarized. However, under elevated P_{CO_2} conditions (Bi), the direction of the ion flow is reversed, causing the neuron to become depolarized (adapted from Tresguerres and Hamilton, 2017). The gill is one of the primary sites of acid–base regulation in fishes; during current-day conditions (Aii), sodium-potassium adenosine triphosphatase (Na^+/K^+-ATPase; NKA) facilitates the excretion of Cl^-, and the transepithelial potential then drives Na^+ secretion, which results in net NaCl secretion. However, during times of elevated P_{CO_2} (Bii), CO_2 may enter ionocytes (see Glossary) from the blood, where carbonic anhydrase (CA) then hydrates this into H^+ (which is secreted in exchange for Na^+) and bicarbonate (HCO_3^-), which is transported into the blood with Na^+; however, this ionocyte model has not yet been verified in marine fishes (adapted from Tresguerres and Hamilton, 2017). Finally, at the red muscle (Aiii), the mechanism whereby oxygen (O_2) is released to the tissues during ambient conditions may be similar to O_2 transport during elevated P_{CO_2}, albeit to a greater degree (Biii); an acidosis at the tissues or in the plasma results in CO_2 moving into the RBCs, where it is catalyzed via CA to form H^+ and HCO_3^-, the former of which binds to haemoglobin (Hb), and O_2 is released. During a mild acidosis, sodium proton exchange (NHE) may play a role in removing H^+ from the RBCs in exchange for Na^+, which, with the combination of anion exchange (AE) removing HCO_3^- in exchange for Cl^-, results in maintenance of intracellular pH. However, if the acidosis is profound (e.g. during times of elevated environmental P_{CO_2}) and/or catecholamines are released, a βNHE may be activated, thus magnifying this process. Regardless, if the H^+ removed from the RBCs is catalyzed with HCO_3^- via plasma-accessible CA (e.g. potentially bound to red muscle endothelial cells), the CO_2 formed will move into the RBCs, re-acidify it, and further promote O_2 release from the Hb (adapted from Rummer et al., 2013b). KC, K^+ channel; NKCC, $Na^+/K^+/2Cl^-$ cotransporter; CFTR, cystic fibrosis transmembrane conductance regulator. Fish illustrations by Erin Walsh.

for exposure periods, types of ecosystems, families of fishes, fish age, CO_2 levels and consequences for metabolic performance.

Strategy underpinning the review of literature
Search engines and terms
Our aim was to identify peer-reviewed articles that observed metabolic responses, specifically responses at the level of oxygen

uptake and AS, in fishes exposed to elevated P_{CO_2}. It was known *a priori* that there exists a range of grades of AS in response to elevated P_{CO_2}; therefore, a literature review using techniques typical of a systematic review was completed (Haddaway et al., 2015a). The search strategy used included a Boolean string in Thomson Reuters Web of Science: ('fish' OR 'teleost') AND ('oxygen consumption' OR 'aerobic scope' OR 'MO$_2$') AND ('hypercapnia' OR 'low pH'

OR 'elevated CO_2' OR 'ocean acidification' OR 'pH' OR 'CO_2' OR 'carbon dioxide'); a search of the terms ('aerobic scope' AND 'fish' AND 'carbon dioxide') and ('oxygen consumption' AND 'fish' AND 'carbon dioxide') in Google Scholar was also made and the first 80 pages were reviewed for appropriate papers (Haddaway et al., 2015b).

All studies that investigated the effect of elevated P_{CO_2} on oxygen uptake in fishes were accessed and reviewed. Studies that were immediately excluded based on title alone were those that were conducted in terrestrial environments or on non-fish species, unavailable, syntheses/reviews, or not related to CO_2. Studies that did not specifically investigate the effects of elevated water P_{CO_2} or were not peer reviewed (e.g. academic theses) were excluded. Studies were then reviewed to determine the 'type' of acidification the study used to alter water pH.

The following information was extracted from each paper: (1) pH range, (2) P_{CO_2} range, (3) species assessed, (4) life stage, (5) length of P_{CO_2} exposure, (6) ecosystem (i.e. marine or freshwater) and (7) type and response of oxygen uptake (i.e. AS, minimum and/or maximum metabolic rate, etc.). Fish were classified as adults unless otherwise specified. Multiple species tested in one study were analyzed separately. Additionally, multiple P_{CO_2} treatments were separated (i.e. fluctuating versus constant). If multiple variables were observed (e.g. temperature and CO_2), only the results exclusively dealing with manipulated P_{CO_2} were compared with control conditions. If multiple exposure periods were examined in one study, these were analyzed individually. Resting and minimum oxygen uptake rates were grouped for analyses and used as estimates of standard metabolic rates (SMRs). Similarly, active and maximum oxygen uptake rates were grouped for analyses and used as estimates of maximum metabolic rates (MMR). Finally, multiple P_{CO_2} exposures were separated for analyses, and mmHg was approximated to µatm using the formula:

$$\left(\frac{X \ \text{mm Hg}}{1}\right) \times \left(\frac{1 \ \text{atm}}{760 \ \text{mm Hg}}\right) \times \left(\frac{1 \ \mu\text{atm}}{10^{-6}\text{atm}}\right) = \mu\text{atm} . \quad (1)$$

The P_{CO_2} level of 2000 µatm was chosen as the AA target. This level is often chosen as the upper limit for OA-targeted marine studies, but we are lacking predictions in freshwater systems, aside from large systems predicted to mirror OA elevations (Phillips et al., 2015). Thus, until we have better predictions for freshwater systems, some of which we know already experience 1000 µatm, 2000 µatm (AA) will be used here for future conditions in both freshwater and marine systems. All data are reported as means±s.e.m.

Log-transformed response ratio
The effect of acidification on AS was calculated as the log-transformed response ratio (lnRR), and each SMR and MMR was used to calculate each reported instance of AS. The lnRR is the ratio of the mean effect of the acidification treatment compared with the mean effect in a control treatment (Hedges et al., 1999). The overall mean effect was calculated for each response variable (AS and each respective oxygen uptake rate, SMR and MMR) by weighting each individual lnRR by the inverse of the sum of its sampling variance and the between-experiment variance, and then calculating the weighted mean (i.e. random effects meta-analysis; Hedges and Olkin, 1985). All calculations were performed in Rstudio (version 1.0.136, https://www.rstudio.com/) using the metafor package (according to Viechtbauer, 2010).

Results
Literature search
Over 238 results were assessed, and 36 studies were identified as having investigated the effects of elevated P_{CO_2} on oxygen uptake rates of fishes (Table S1). When species, P_{CO_2} levels and exposure times within each study were separated, 93 reports were examined across the 36 studies. Of the included studies, the majority (89.2%) reported SMR, 44.1% reported MMR and fewer studies (28.0%) reported AS.

Of the 26 instances where responses to elevated P_{CO_2} at the level of AS were examined, the majority (73.1%) reported no effect of elevated P_{CO_2} on AS compared with control conditions, whereas 15.3% reported a decrease and 11.5% reported an increase in AS. When the reports were distinguished between those above or below the P_{CO_2} levels expected by 2300 owing to increased AA, 18 instances reported AS results, 14 reported no change, three observed increases and one observed a decrease in AS as a result of elevated P_{CO_2}. Of the 29 reports not focused on climate-change-relevant P_{CO_2} levels, only eight species in total were examined, with the majority (58.6%) coming from marine systems.

To date, only two studies have examined the effects of fluctuating (versus constant or elevated) P_{CO_2} levels on fishes (Methling et al., 2013; Ou et al., 2015). The range of constant P_{CO_2} levels that have been used to examine the effects on oxygen uptake in fishes so far has spanned 260 to 25,000 µatm (3975.0±627.5 µatm). Most of these instances were examined in marine systems (78%) spanning P_{CO_2} levels of 600 to 25,000 µatm (3757.3±750.4 µatm). In freshwater systems (22% of all the O_2 uptake rate data), the P_{CO_2} levels under which fish have been examined have ranged from 260 to 19,000 µatm (4747.9±1040.7 µatm).

Most species studied were from the class of ray-finned fishes (i.e. class Actinopterygii; see Glossary); however, 11 of the total collated data points were for chondrichthyan species (i.e. class Chondrichthyes; see Glossary). Of the 11 instances, five reported AS, and of these, three reported no effect of elevated P_{CO_2} and two reported an increase in AS.

Finally, the majority of fishes examined were either juvenile or adult stages (43.0% for each). Few studies reported metabolic responses to elevated P_{CO_2} in larvae or embryos (5.4 and 8.6%, respectively). Furthermore, there were seven reports for adult, 11 for juvenile and two for embryonic fishes in terms of the effects of elevated P_{CO_2} on AS, and the overall response was that of no effect. There was only one reported increase and one reported decrease in AS in adult fishes, and two increases and one decrease in juveniles.

lnRR
When all respective metabolic performance responses used to calculate each AS were pooled (i.e. SMR and MMR), elevated P_{CO_2} had a significant effect on SMR (i.e. SMR responses were elevated compared with those of controls) but no effect on MMR or AS (Fig. 2).

A biological synthesis
Maintaining performance and tolerating hypercapnia
Most studies examined oxygen uptake rates either while fish were resting (SMR) or during and/or following exercise (MMR). While important, these metrics may only be capturing half of the story (Fig. 2). As revealed from this review process, the least-reported O_2 uptake metric was AS, which can aid in predicting whole-animal performance and fitness. When either SMR or MMR are reported alone, any conclusions drawn may not have whole-animal implications. For instance, the lnRR of SMR collected from
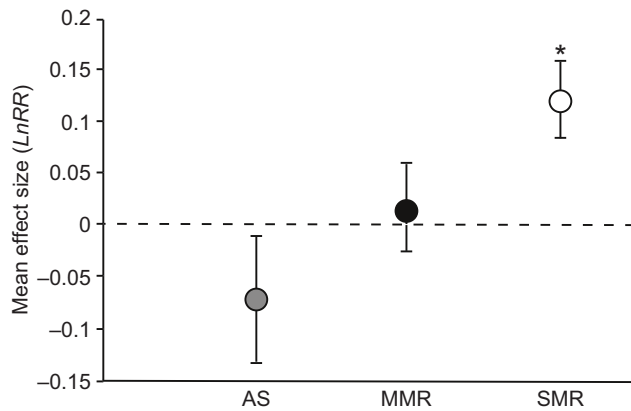
Fig. 2. Differences in the mean effect of elevated P_{CO_2} on aerobic scope (AS; $n=26$), maximum metabolic rates (MMR; $n=26$) and standard metabolic rates (SMR; $n=26$) used to calculate the reported instances of AS of fishes. An asterisk represents a significant difference from zero (*$P<0.05$) (data from Couturier et al., 2013; Di Santo, 2015, 2016; Esbaugh et al., 2016; Gräns et al., 2014; Green and Jutfelt, 2014; Hamilton et al., 2017; McKenzie et al., 2003; Melzner et al., 2009; Methling et al., 2013; Munday et al., 2009; Nadler et al., 2016; Ou et al., 2015; Rummer et al., 2013a; Tirsgaard et al., 2015).

reports used to calculate AS indicates that fish have higher SMR when exposed to elevated P_{CO_2}, suggesting they require more energy for basic maintenance and are ultimately negatively impacted by elevated P_{CO_2}; however, when MMR and AS are examined from the same datasets, there is no effect of acidification (Fig. 2). If SMR had been reported alone, the conclusions drawn from the data would have suggested that the energy budget of fishes exposed to elevated P_{CO_2} is negatively affected. However, when AS is examined alone, there seems to be no effect, suggesting that fish are unaffected in terms of whole-animal performance and fitness. The lack of data examining the whole picture makes it difficult to draw whole-animal performance conclusions and/or predictions that are key when considering how fish populations will be affected by future acidification scenarios in aquatic ecosystems. We conclude that observing one oxygen uptake metric alone may not be enough.

Of all 26 instances reporting AS responses to elevated P_{CO_2}, only four reported decreased AS (15.4%), all of which were in marine species, but only one within the range of future predicted conditions arising owing to OA – that for the cardinalfish *Ostorhinchus cyanosoma* (Munday et al., 2009). The other three that reported decreases in AS were all different species and involved studies using very high (e.g. 2800, 8000 and 9200 µatm) P_{CO_2} levels (Hamilton et al., 2017; Methling et al., 2013; Tirsgaard et al., 2015, respectively). Thus, in the remaining studies examining AS responses to elevated P_{CO_2} (84.6%), performance has either been maintained (73.1%) or increased (11.5%) compared with controls for 12 different species of fish. Additionally, exposure time did not seem to factor in to AS responses. The exposure periods ranged from less than a day to a year for all of the AS measurements. The increased AS responses compared with those of controls varied from 17 to 224 days of exposure, and the decreased AS responses compared with controls arose from exposures ranging from 4 to 112 days. This trend could indicate that compensating for the acidosis ensuing from these elevated P_{CO_2} levels is not a significant additional metabolic cost for fish, and no increase in energy is required to maintain aerobic processes. This may also help support an earlier prediction that fish are relatively tolerant to hypercapnia – in terms of metabolic performance (Heuer and Grosell, 2014) – and

are therefore able to maintain whole-animal performance, even under future higher AA levels. The majority of fish species examined thus far were able to maintain performance during acidosis, suggesting that fishes are using one or more mechanism to achieve this. One explanation for this tolerance or insensitivity to elevated P_{CO_2} could be at the mechanistic level, where Root effect Hbs coupled with plasma-accessible CA can enhance oxygen delivery to select tissues during a mild acidosis (Rummer and Brauner, 2011; Rummer et al., 2013a,b).

Maintaining performance in elasmobranch fishes

Although the Root effect has been relatively well studied in teleost fishes, elasmobranchs are not known to possess these unique Hbs, which has been evidenced by the mere <10% reduction in Hb–O$_2$ saturation in elasmobranchs under an acidosis when compared with the >40% reduction noted for teleosts (Berenbrink et al., 2005). Thus, Root effect Hbs would not be capable of playing a role in enhancing or maintaining O$_2$ delivery in elasmobranchs during times of elevated P_{CO_2}. However, in all studies examining the effects of elevated P_{CO_2} on elasmobranchs to date, AS was maintained (83.3%) or increased (16.7%) (Di Santo, 2015, 2016; Green and Jutfelt, 2014). Although there are minimal data – only five reports considered the effects of elevated P_{CO_2} on AS – the evidence that is available suggests that, similar to teleost fishes, elasmobranchs can maintain or even enhance performance when exposed to likely future OA conditions. We know that elasmobranchs do not possess Root effect Hbs (Berenbrink et al., 2005), yet they retain the ability to maintain or increase performance. This may suggest that elasmobranchs possess a different mechanism that allows them to maintain performance when P_{CO_2} is elevated, and it is possible that some basal lineages of living ray-finned fishes may use this as well, as evidenced by their minimal decrease in Hb–O$_2$ saturation with a decrease in pH, suggesting a modest to non-existent Root effect (Berenbrink et al., 2005). Identifying these mechanisms becomes important as these populations are predicted to experience elevations in P_{CO_2} owing to AA, and this lack of knowledge on how these important meso- and apex predators (see Glossary) are able to maintain performance under acidified conditions points to a need for further research to identify the underlying mechanism.

Other mechanisms underpinning maintained performance

It is important to point out that the mechanism associated with Root effect Hbs and plasma-accessible CA represents just one explanation for increased O$_2$ delivery to tissues in teleost fishes during exposure to elevated P_{CO_2}. Although often implied, no studies to date have actually linked this mechanism directly to maintained O$_2$ uptake and/or performance metrics. Other proposed mechanisms to explain maintained O$_2$ uptake metrics may have a genetic basis. A recent study by Schunter et al. (2018) suggests that transcriptional changes in the brain during acidification may be linked to the oxidative stress response [e.g. stanniocalcin (*stc2*)]. Additionally, an increase in brain fibroblast growth factor 1 (*fgf1*) can promote blood glucose reduction. This aids in the ability of fishes to rebalance gluconeogenesis and glucose homeostasis, which the authors suggest is key to adapting to new environmental conditions (Schunter et al., 2018). Furthermore, the physiological changes that occur at the branchial level are also crucial in determining the efficiency of gas exchange, ion balance and acid–base regulation, all of which underpin O$_2$ transport. Specifically, both the diffusion distance of the respiratory epithelium and the permeability of the gas through the epithelium can impact gas exchange (Perry et al., 2009). Esbaugh et al. (2016) suggest that, when fish are exposed to hypercapnic

conditions, branchial plasticity can reduce diffusion distance, enhancing CO_2 extrusion. For example, the branchial diffusion distance was reduced by 32% in fish exposed to elevated P_{CO_2} when compared with control fish. Another mechanism that can aid in maintaining O_2 transport is increased cardiac flow-generating capacity during exposure to acidic conditions (Gräns et al., 2014). The mechanisms responsible for enhanced cardiac pumping capacity in fishes acclimated to elevated P_{CO_2} conditions remain unknown (Gräns et al., 2014); however, this finding emphasizes that more research is needed to tease apart the mechanisms fish use to maintain oxygen transport during an acidosis.

Freshwater fishes: trawling for data

Although elasmobranchs have been insufficiently studied in terms of the effects of elevated P_{CO_2} (Rosa et al., 2017), a more obvious data gap is for the freshwater fishes. Only three data points from one study on one species showed the response of AS to elevated P_{CO_2} in freshwater systems. Thus, in addition to lacking predictions of how the P_{CO_2} of freshwater systems will change in the future, studies investigating how possible elevations will affect the whole-organism performance of these fishes, which would have important fitness implications, are lacking. Furthermore, in all freshwater reported results (20), only five reports were attained for non-adult fish and in only six instances were freshwater fishes exposed to elevated P_{CO_2} conditions for more than 2 days. Collectively, this information further highlights the need for firm predictions as to how P_{CO_2} will change in freshwater systems in the future; we urge that studies should focus on an array of age classes and use an array of exposure times.

Fishing for facts: embryology escapes the net

Although exceptions exist, development and growth of embryos seems to be generally unaffected by P_{CO_2} (Bignami et al., 2013; Frommel et al., 2013; Heuer and Grosell, 2014; Munday et al., 2011). However, data on O_2 uptake metrics are also lacking for embryonic and larval marine fishes exposed to elevated P_{CO_2}. Adding to our knowledge of how elevated P_{CO_2} affects O_2 uptake metrics in early-life-stage fish is especially important as these life stages are some of the most sensitive to environmental perturbations (Ishimatsu et al., 2005). Additionally, the role of Root effect Hbs in O_2 delivery has predominantly been examined in adult fishes, largely salmonids (Alderman et al., 2016; Berenbrink et al., 2005, 2011; Rummer and Brauner, 2015; Rummer et al., 2013b; Waser and Heisler, 2005). However, larval pink salmon decrease MMR upon exposure to elevated P_{CO_2} (Ou et al., 2015), which suggests that there is less energy available for maximum performance, which has implications for predator escape, settlement, foraging, etc. Note that this is just one metric – and we previously mentioned the danger of drawing conclusions based on one metric. Ontogenetic shifts may allow salmonids and other fishes to maintain and increase performance under elevated P_{CO_2}. Other metrics such as swimming performance, growth and survival are also important during early development, with wide implications for the structure of fish communities, which could be even more important if there is an ontogenetic shift in performance in response to elevated P_{CO_2}.

Concluding remarks

Based on our meta-review, it is clear that there are many gaps in the literature regarding the metabolic responses of fish to elevated P_{CO_2}. However, the general trend, regardless of species, exposure time, CO_2 level and ontogeny, is that performance is largely maintained, with over 60% of all the reports for each trait (SMR, MMR and AS) showing no effect upon exposure to elevated P_{CO_2}.

Despite there being only a few empirical studies focused on the AS responses of fishes to AA-relevant P_{CO_2} levels, the only increases in AS observed were upon exposure to the range encompassing 900 to 2000 µatm, whereas the majority of the decreases in AS were upon exposure to levels exceeding 2000 µatm. This suggests that fish have a mechanism to maintain and even increase AS under future higher levels of AA; however, once those P_{CO_2} thresholds are exceeded, metabolic performance could start decreasing. This is not to say, however, that fish will be unable to use phenotypic plasticity and transgenerational acclimation and, ultimately, adapt to increasing P_{CO_2} over time (Donelson et al., 2011, 2012; Miller et al., 2012).

This Review highlights many gaps in the literature that need to be addressed, but furthermore, serves to reveal a current consensus of studies to date that have found minimal adverse effects of AA on AS. Additionally, we have put forward a hypothesis that could explain how fish maintain aerobic performance during an acidosis. O_2 uptake rates are often used as a proxy for metabolic rates, and, from these, we can estimate the amount of energy available to an animal under different environmental conditions. The amount of energy available to an animal can affect all life processes (e.g. growth, feeding, digestion, swimming, avoiding predators, reproduction, etc.), and thus, if altered during exposure to a certain environmental condition, there can be dramatic implications for future populations. Additionally, there has been recent emphasis on determining 'winners' and 'losers' during challenging environmental conditions and the traits responsible. These traits have the potential to make an organism more successful and thus increase their survival and reproductive success. If these traits are heritable, the population will start changing.

Variations in behaviours upon exposure to elevated P_{CO_2} have been linked to differential expression of genes controlling circadian rhythm (Schunter et al., 2016). Offspring of behaviourally tolerant parents displayed a flexibility of gene expression compared to offspring of P_{CO_2} sensitive parents, allowing them to phase shift their circadian clock and avoid detrimental reactions to elevated P_{CO_2} conditions. Thus, there is evidence that individual variation in CO_2 sensitivity could facilitate adaptation of fishes and has the potential to influence populations in the future (Schunter et al., 2016). This combination of mechanistic and behavioural research is crucial for predicting the future fate of fish populations. In terms of oxygen uptake, studies on Root effect Hbs have been almost exclusively mechanistic, and the studies on oxygen uptake, swimming performance and behaviour in response to climate change have largely been separate from mechanistic work. The variability of traits that mechanistic research can illuminate can help highlight the traits that selection will be acting on that will drive future adaptations and/or populations. Indeed, collaborative research combining molecular, physiological and ecological approaches will allow us to better predict how fish communities will be affected in the future by OA, and will aid the identification of the systems or species that will be able to adapt and the species that are vulnerable and in need of management.

Supplementary information
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Table S1. Summary of the individual data points from experimentally based studies on the impacts of aquatic acidification (AA) on oxygen consumption of fishes. Abbreviations: SMR, standard/resting metabolic rate; MMR, maximum metabolic rate; AS, aerobic scope. Symbols: X, no effect; ↓, decrease; ↑, increase.

Fish species	Life stage	~pCO$_2$	Habitat	SMR	MMR	AS	Reference
Anguilla anguilla	adult	7894.7	marine	X	↓	↓	(Methling et al., 2013)
Gadus morhua	adult	9200.0	marine	X	↓	↓	(Tirsgaard et al., 2015)
Scophtalmus maximus	juvenile	15000.0	marine	X			(Stiller et al., 2015)
Scophtalmus maximus	juvenile	15000.0	marine	X			(Stiller et al., 2015)
Scophtalmus maximus	juvenile	15000.0	marine	X			(Stiller et al., 2015)
Scophtalmus maximus	juvenile	15000.0	marine	X			(Stiller et al., 2015)
Scophtalmus maximus	juvenile	25000.0	marine	X			(Stiller et al., 2015)
Scophtalmus maximus	juvenile	25000.0	marine	X			(Stiller et al., 2015)
Scophtalmus maximus	juvenile	25000.0	marine	↓			(Stiller et al., 2015)
Scophtalmus maximus	juvenile	25000.0	marine	↓			(Stiller et al., 2015)
Anguilla anguilla	adult	fluctuating	marine	X	↓	X	(Methling et al., 2013)
Symphodus ocellatus	embryos	1000.0	marine	↑			(Cattano et al., 2016)
Anguilla anguilla	adult	5263.2	freshwater	X			(Cruz-Neto and Steffensen, 1997)
Anguilla anguilla	adult	10526.3	freshwater	X			(McKenzie et al., 2002)
Acipenser transmontanus	juvenile	7894.7	freshwater	↓			(Baker and Brauner, 2012)
Anguilla anguilla	adult	7894.7	freshwater	X			(McKenzie et al., 2002)
Acipenser transmontanus	juvenile	5921.1	freshwater	↓			(Baker and Brauner, 2012)
Anguilla anguilla	adult	5921.1	freshwater	X	X	X	(McKenzie et al., 2003)
Anguilla anguilla	adult	5263.2	freshwater	X			(McKenzie et al., 2002)
Acipenser transmontanus	juvenile	3947.4	freshwater	↑			(Baker and Brauner, 2012)
Anguilla anguilla	adult	3947.4	freshwater	X	X	X	(McKenzie et al., 2003)
Anguilla anguilla	adult	2631.6	freshwater	X			(McKenzie et al., 2002)
Acipenser transmontanus	juvenile	1973.7	freshwater	X			(Baker and Brauner, 2012)
Anguilla anguilla	adult	1973.7	freshwater	X	X	X	(McKenzie et al., 2003)

Salvelinus fontinalis	adult	260.0	freshwater		↓		(Basu, 1959)
Catostomus commersoni	adult	260.0	freshwater		↓		(Basu, 1959)
Ameiurus nebulosus	adult	260.0	freshwater		↓		(Basu, 1959)
Cyprinus carpio	adult	260.0	freshwater		↓		(Basu, 1959)
Carassius auratus	adult	260.0	freshwater		↓		(Basu, 1959)
Hemiscyllium ocellatum	adult	600.0	marine	X			(Heinrich et al., 2014)
Trematomus bernacchii	juvenile	650.0	marine	X			(Davis et al., 2016)
Trematomus bernacchii	juvenile	650.0	marine	X			(Davis et al., 2016)
Trematomus bernacchii	juvenile	650.0	marine	X			(Davis et al., 2016)
Chromis viridis	adult	750.0	marine	X	X	X	(Nadler et al., 2016)
Sebastes caurinus	juvenile	750.0	marine	X	X	X	(Hamilton et al., 2017)
Sebastes mystinus	juvenile	750.0	marine	X	X	X	(Hamilton et al., 2017)
Notothenia rossii	adult	850.0	marine	X			(Strobel et al., 2012)
Pomacentrus moluccensis	juvenile	860.0	marine	X	X		(Couturier et al., 2013)
Pomacentrus ambioensis	juvenile	860.0	marine	X	↑		(Couturier et al., 2013)
Pomacentrus moluccensis	juvenile	860.0	marine		X		(Couturier et al., 2013)
Pomacentrus ambioensis	juvenile	860.0	marine		↑		(Couturier et al., 2013)
Pomacentrus ambioensis	juvenile	860.0	marine		↑		(Couturier et al., 2013)
pseudochromis fuscus	adult	860.0	marine	X	X	X	(Couturier et al., 2013)
Hemiscyllium ocellatum	adult	880.0	marine	X			(Heinrich et al., 2014)
Acanthochromis polyacanthus	adult	946.0	marine	↓	↑	↑	(Rummer et al., 2013)
Scyliorhinus canicula	adult	990.0	marine	X	X	X	(Green and Jutfelt, 2014)
ambligliphiodon melanopus	juvenile	1000.0	marine	X			(Miller et al., 2012)
Chromis viridis	adult	1000.0	marine	X	X	X	(Nadler et al., 2016)
ostorhinchus doederleini	adult	1000.0	marine	X	X	X	(Munday et al., 2009)
ostorhinchus cyanosoma	adult	1000.0	marine	↑	X	↓	(Munday et al., 2009)
Sciaenops ocellatus	juvenile	1000.0	marine	X	X	X	(Esbaugh et al., 2016)
Hippoglossus hippoglossus	juvenile	1000.0	marine	X	X	↑	(Gräns et al., 2014)
Trematomus bernacchii	adult	1000.0	marine	X			(Enzor et al., 2013)

Trematomus bernacchii	adult	1000.0	marine	X			(Enzor et al., 2013)
Trematomus hansoni	adult	1000.0	marine	X			(Enzor et al., 2013)
Trematomus hansoni	adult	1000.0	marine	X			(Enzor et al., 2013)
Pagothenia borchgrevinki	adult	1000.0	marine	X			(Enzor et al., 2013)
Pagothenia borchgrevinki	adult	1000.0	marine	X			(Enzor et al., 2013)
Trematomus bernacchii	adult	1000.0	marine	↑			(Enzor et al., 2013)
Trematomus newesi	adult	1000.0	marine	↑			(Enzor et al., 2013)
Trematomus bernacchii	juvenile	1050.0	marine	X			(Davis et al., 2016)
Trematomus bernacchii	juvenile	1050.0	marine	X			(Davis et al., 2016)
Trematomus bernacchii	juvenile	1050.0	marine	X			(Davis et al., 2016)
Leucoraja erinacea	juvenile	1100.0	marine	X	X	X	(Di Santo, 2016)
Leucoraja erinacea	juvenile	1100.0	marine	X	↑	↑	(Di Santo, 2016)
Leucoraja erinacea	embryos	1100.0	marine	X	X	X	(Di Santo, 2015)
Leucoraja erinacea	embryos	1100.0	marine	↑	↑	X	(Di Santo, 2015)
Gadus morhua	adult	1200.0	marine	X			(Kreiss et al., 2015)
Chiloscyllium punctatum	embryos	1400.0	marine	↓			(Rosa et al., 2014)
Chiloscyllium punctatum	juvenile	1400.0	marine	X			(Rosa et al., 2014)
Chiloscyllium punctatum	embryos	1400.0	marine	X			(Rosa et al., 2014)
Chiloscyllium punctatum	embryos	1400.0	marine	X			(Rosa et al., 2014)
Pomacentrus ambioensis	juvenile	1400.0	marine		X		(Couturier et al., 2013)
Hippocampus guttulatus	adult	1400.0	marine	X			(Faleiro et al., 2015)
Oncorhynchus gorbuscha	larvae	1600.0	marine	X	↓		(Ou et al., 2015)
solea senegalensis	larvae	1600.0	marine	X			(Pimentel et al., 2015)
solea senegalensis	larvae	1600.0	marine	↓			(Pimentel et al., 2015)
Coryphaena hippurus	larvae	1600.0	marine	↓			(Pimentel et al., 2014)
Girella laevifrons	juvenile	1600.0	marine	X			(Benítez et al., 2017)
Sebastes caurinus	juvenile	1900.0	marine	X	X	X	(Hamilton et al., 2017)
Sebastes mystinus	juvenile	1900.0	marine	X	X	X	(Hamilton et al., 2017)
Oncorhynchus gorbuscha	embryos	2000.0	freshwater	X	X		(Ou et al., 2015)

Gadus morhua	adult	2200.0	marine	X			(Kreiss et al., 2015)
Pomacentrus ambioensis	juvenile	2400.0	marine		X		(Couturier et al., 2013)
Sebastes mystinus	juvenile	2800.0	marine	X	X	X	(Hamilton et al., 2017)
Sebastes caurinus	juvenile	2800.0	marine	X	X	↓	(Hamilton et al., 2017)
Sciaenops ocellatus	juvenile	5000.0	marine	X			(Ern and Esbaugh, 2016)
Gadus morhua	juvenile	5800.0	marine	X	X	X	(Melzner et al., 2009)
Zoarces viviparus	adult	10000.0	marine	X			(Deigweiher et al., 2008)
Oncorhynchus gorbuscha	larvae	fluctuating	marine	X	X		(Ou et al., 2015)
Sander lucioperca	adult	9500.0	freshwater	X			(Steinberg et al., 2017)
Sander lucioperca	adult	19000.0	freshwater	↓			(Steinberg et al., 2017)
Gadus morhua	embryos	1100.0	marine	↑			(Dahlke et al., 2017)

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